

Modulation of Receptor Signaling and Functional Selectivity in Neutrophils

Akademisk avhandling

som för avläggande av medicine doktorsavhandling vid Sahlgrenska akademien vid Göteborgs universitet kommer att offentlig försvaras i föreläsningssalen, våning 3, Guldhedsgatan 10A, Göteborg

Fredag den 17 november 2017 kl 09:00

av

Michael Gabl

Fakultetsopponent: Jean-Yves Springael, Ph.D., tenured lecturer
Institute of Interdisciplinary Research in human and molecular Biology (IRIBHM)
Université Libre de Bruxelles, Brussels

- I Michael Gabl, Malene Winther, Sarah Line Skovbakke, Johan Bylund, Claes Dahlgren, Huamei Forsman
A Pepducin Derived from the Third Intracellular Loop of FPR2 Is a Partial Agonist for Direct Activation of This Receptor in Neutrophils But a Full Agonist for Cross-Talk Triggered Reactivation of FPR2
PLoS One, 2014, 9(10):e109516

- II Michael Gabl, Malene Winther, Amanda Welin, Anna Karlsson, Tudor Oprea, Johan Bylund, Claes Dahlgren, Huamei Forsman
P2Y₂ receptor signaling in neutrophils is regulated from inside by a novel cytoskeleton-dependent mechanism
Experimental Cell Research, 2015, 336(2):242-52

- III Michael Gabl, André Holdfeldt, Malene Winther, Tudor Oprea, Johan Bylund, Claes Dahlgren, Huamei Forsman
A pepducin designed to modulate P2Y₂R function interacts with FPR2 in human neutrophils and transfers ATP to an NADPH-oxidase-activating ligand through a receptor cross-talk mechanism
Biochimica et Biophysica Acta (BBA) – Molecular Cell Research, 2016, 1863(6 Pt A):1228-37

- IV Michael Gabl, Andre Holdfeldt, Martina Sundqvist, Jalal Lomei, Claes Dahlgren, Huamei Forsman
FPR2 signaling without β -arrestin recruitment alters the functional repertoire of neutrophils
Biochemical Pharmacology, 2017, 10.1016/j.bcp.2017.08.018 (*in press*)



UNIVERSITY OF GOTHENBURG

Modulation of Receptor Signaling and Functional Selectivity in Neutrophils

Michael Gabl

Department of Rheumatology and Inflammation Research, Institute of Medicine,
University of Gothenburg, Gothenburg, Sweden, 2017

Abstract: Neutrophils are important effector cells of the innate immune system and in the regulation of inflammation. Many of their functions, such as chemotactic migration, secretion of granule constituents and activation of the oxygen radical-producing NADPH-oxidase, are regulated by cell surface receptors. The formyl peptide receptors (FPRs), the ATP receptor (P2Y₂R) and the receptor for platelet activating factor (PAFR) belong to the large family of G-protein coupled receptors (GPCRs) and, amongst other receptors, enable neutrophils to sense and respond to host- and pathogen-derived danger signals. Therefore, any regulatory imbalance in GPCR signaling can potentially contribute to the development of severe infections or autoimmune/inflammatory diseases.

The work presented in this thesis is focused on basic GPCR-signaling mechanisms in human neutrophils with the aim to generate new knowledge that could be of value for future GPCR-based drug development. To answer the scientific questions raised, numerous cell-biology-based experimental methods were applied, including measurements of neutrophil intracellular calcium release, super-oxide production, degranulation, cell migration and cytoskeleton-mediated receptor regulation.

The functional responses triggered by GPCRs expressed by neutrophils can be modulated in various ways at the level of receptors/ligand interaction, in dependence of other GPCRs, as well as at the signaling level. Both FPR2 and P2Y₂R have been shown to be able to exert functional selective signaling through distinct regulatory mechanisms. An FPR2-specific synthetic lipopeptide allosteric modulator was identified as a biased agonist that does not induce recruitment of β -arrestin or chemotactic migration and exhibits oppositional efficacies for direct FPR2 activation and receptor cross-talk-mediated signaling. Functional selectivity linked to the P2Y₂R is not related to biased agonism but instead emerges from an endogenous actin cytoskeleton-dependent regulatory mechanism which selectively inhibits the signals that lead to the generation of oxygen radicals, while leaving other signaling pathways unaffected.

In conclusion, this thesis adds new knowledge to the field of neutrophil receptor biology and provides novel insights into the modulation of basic GPCR signaling mechanisms with intend to contribute to strategies for future drug design and treatment of inflammatory disorders and disease.

Keywords: neutrophils, G-protein coupled receptors, reactive oxygen species, ligands, functional selectivity, allosteric modulation

ISBN: 978-91-629-0298-8 (print), 978-91-629-0299-5 (pdf)